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Vasoprotective effects of an endothelin receptor antagonist in ovariectomized female rats

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ABSTRACT

Aims: The effects of hormone replacement therapy with estrogen on cardiovascular disease in postmenopausal women are still controversial. In the present study, we examined the effects of an endothelin (ET) receptor antagonist (ERA) and/or angiotensin receptor blocker (ARB) on neointimal formation following vascular injury in ovariectomized (OVX) female rats.

Main methods: Female rats were divided into intact female and OVX groups. The right carotid artery was subjected to balloon injury, and harvested 2 weeks later.

Key findings: In the intact female groups, treatment with ARB (L-158809; 1 mg/kg/day) for two weeks after the injury significantly decreased neointimal formation, whereas treatment with the ERA (J-104132; 10 mg/kg/day) did not affect neointimal formation. On the other hand, the ERA markedly decreased neointimal formation after the injury in the OVX groups; however, neointimal formation was not significantly improved by the ARB treatment. In addition, the combined treatment with 17β-estradiol (20 μg/kg/day) or the ERA and ARB markedly suppressed neointimal formation after the balloon injury in the OVX groups, whereas no combinational effects were observed due to the combined treatment with 17β-estradiol and the ERA.

Significance: These results suggest that ERAs have estrogen-like vasoprotective effects on neointimal formation following balloon injury in OVX rats. ERAs may be useful as an alternative therapy to prevent vascular disease in postmenopausal women.

Introduction

The incidence of cardiovascular disease has been shown to be lower in women prior to menopause than in men and postmenopausal women (Mahendru and Morris, 2013; Maranon and Reckelhoff, 2013). The mechanisms underlying these lower risks in premenopausal women have not been elucidated completely, but can at least be partly explained by the vasoprotective effects of estrogen (Bernelot Moens et al., 2012; Farhat et al., 1996; Pare et al., 2002). Previous studies showed that estrogen increased endothelial nitric oxide production and suppressed smooth muscle proliferation/migration, oxidative stress, and inflammation in the vessels (Florian et al., 2004; Miller et al., 2003; Tolbert et al., 2001). Several clinical studies reported the beneficial effects of estrogen replacement therapy (ERT) on the risk of cardiovascular diseases in postmenopausal women (Grady et al., 1992; Walsh et al., 1991). In contrast, other clinical trials, including the Heart and Estrogen–Progestin Replacement Study (HERS) and Women's Health Initiative (WHI) Clinical Trial and observational study, found no beneficial effects of ERT (Hulley et al., 1998; Rossouw et al., 2002). Thus, the efficacy of ERT on cardiovascular disease is still controversial in clinical settings. Elucidating the mechanisms of estrogen-induced vasoprotective effects and alternative estrogen therapies in postmenopausal women in more detail remains a critical issue.

Endothelin (ET)-1 exhibits potent vasoconstriction and mitogenic effects on vascular smooth muscle cells (VSMCs) (Kirchengast and Munter, 1998; Kitada et al., in press; Ohkita et al., 2012). The ET-1/ET receptor system-induced proliferation of VSMCs and neointimal formation are known to be involved in the development of vascular diseases such as atherosclerosis, restenosis, and hypertension or diabetes-induced arterial hypertrophy; therefore, endothelin receptor antagonists (ERAs) may be used as vasoprotective drugs to treat vascular diseases (Kirchengast and Munter, 1998; Kitada et al., in press).

Previous studies showed that the ET-1/ET receptor system is closely related to the sex differences associated with cardiovascular diseases (Kawanishi et al., 2007; Kitada et al., 2011; Lekontseva et al., 2010; Tostes et al., 2008). For example, plasma ET-1 concentrations were shown to be lower in women than in men and old women had higher plasma ET-1 levels than young women (Best et al., 1998; Maeda et al., 2003; Miyauchi et al., 1992). In addition, the administration of

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17β-estradiol has been suggested to decrease plasma ET-1 levels in postmenopausal women (Best et al., 1998). Moreover, previous studies reported that estrogen could regulate the production and function of ET-1 as well as ET receptor expression in the cardiovascular system (Dubey et al., 2001; Kitada et al., in press; Lekontseva et al., 2010; Pedersen et al., 2008; Tostes et al., 2008). We previously demonstrated that ET receptor-mediated ET-1 actions were stronger in the vascular lesion sites of males than in those of intact females (Kitada et al., 2011). These results indicate that the inhibitory effects of estrogen on the ET system contribute to the mechanisms underlying the sex differences associated with cardiovascular diseases. Thus, the vascular ET-1/ET receptor system may be augmented and contribute to the increase in the incidence of cardiovascular events in postmenopausal women because of estrogen deficiencies. Taken together, ERAs may be a new target to replace estrogen and become an effective therapy for reducing the risk of cardiovascular diseases after menopause.

In the present study, we hypothesized that an ERA may effectively prevent the development of vascular lesions in OVX female rats as a replacement for estrogen. To confirm this hypothesis, we examined the vasoprotective effects of an ERA on balloon injury-induced neointimal formation in intact and ovariectomized (OVX) female rats and compared these effects with those of angiotensin receptor blockade (ARB), which is one of the key vasoprotective drugs used in clinical cases (Hernandez Schulman et al., 2007; Nakashima et al., 2006).

Materials and methods

Animals

Animals were housed in a light-controlled room with a 12-hour light/dark cycle and were allowed *ad libitum* access to food and water. Experimental protocols and animal care methods in the experiments were approved by the Experimental Animal Committee at Osaka University of Pharmaceutical Sciences, and all studies were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Experimental protocols

Female Sprague–Dawley (SD) rats (9 weeks old, Japan SLC, Shizuoka, Japan) were divided into intact female and OVX groups. Ovariectomy or sham surgery was performed under anesthesia using an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (5 mg/kg). Rats underwent balloon injury to the right carotid artery one week after surgery. Some intact female and OVX rats were gavaged with J-104132 (an ET_A/ET_B dual receptor antagonist: 10 mg/kg/day), (+)-(5S,6R,7R)-2-butyl-7-[2-((2S)-2-carboxypropyl)-4-methoxyphenyl]-5-(3,4-methylenedioxyphenyl)cyclopenteno[1,2-b]pyridine-6-carboxylic acid, and/or L-158809 (an AT_1 receptor antagonist: 1 mg/kg/day), 5,7-dimethyl-(2-ethyl-3-[[2′-(1H-tetrazol-5-yl)[1,1′]-biphenyl-4-yl]methyl]-3H-imidazo[4,5-b] pyridine, for 2 weeks starting 12 h after the balloon injury. Furthermore, some OVX rats were treated daily with the subcutaneous administration of 17β-estradiol (20 μg/kg/day) and/or ICI 182,780 (an estrogen receptor inhibitor: 5 mg/kg/day) starting 24 h before the balloon injury, with or without J-104132 or L-158809. The administration of J-104132, L-158809, 17β-estradiol, ICI 182,780, or vehicle was continued until 2 weeks after the balloon injury. The doses of these drugs were determined based on previous studies (Bakir et al., 2000; Huckle et al., 1996; Kitada et al., 2009, 2011). We also determined the effects of these doses of J-104132 and L-158809 in male rats in separate experiments. Relative to the vehicle treatment, J-104132 or L-158809 significantly suppressed the neointima/media ratio to the same extent (0.71 ± 0.09 vs 0.42 ± 0.06 or 0.46 ± 0.06, respectively, $P < 0.05$, $n = 4$ per group). We used the following 12 groups in this study.

1) intact + vehicle
2) intact + ERA
3) intact + ARB
4) OVX + vehicle
5) OVX + ERA
6) OVX + ERA + ICI 182,780
7) OVX + ARB
8) OVX + 17β-estradiol
9) OVX + 17β-estradiol + ICI 182,780
10) OVX + 17β-estradiol + ERA
11) OVX + 17β-estradiol + ARB
12) OVX + ERA + ARB

Balloon injury model

Rats were anesthetized using an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (5 mg/kg), and the right carotid artery was injured with a 2 F Fogarty balloon catheter (Baxter International, Deerfield, IL, USA), as described previously (Kurumazuka et al., 2006). The left carotid artery was not damaged. Rats were sacrificed 2 days and 2 weeks after the balloon injury with a sodium pentobarbital overdose (75 mg/kg), and both the left and right carotid arteries were harvested.

Morphometric analysis

The bilateral carotid arteries 2 weeks after the balloon injury were fixed in 10% formalin, embedded in paraffin, and cut into 4 μm-thick sections. Tissue sections were stained using the Elastica Van Gieson method. Morphometric analysis of each arterial segment was performed with a computer-based Motic Image Plus 2.0 Morphometric system (Shimadzu, Kyoto, Japan). The border of the lumen, internal elastic lamina, and external elastic lamina were traced and the neointimal and medial areas were measured. The ratio of the neointimal to medial area (neointima/media ratio) was calculated by dividing the neointimal area by the medial area.

NADPH-dependent superoxide production

NADPH oxidase activity was measured based on the degree of NADPH-dependent superoxide production in the isolated carotid arteries, as assessed by a lucigenin-enhanced assay. We examined NADPH-dependent superoxide production 2 days after the balloon injury based on our previous study (Kurumazuka et al., 2006). Briefly, both injured (right) and uninjured (left) common carotid arteries were cleared of adherent adipose and loose connective tissue *in situ* and were harvested in ice-cold modified Krebs–HEPES buffer containing (in mmol/L): NaCl 99.01, HEPES 20, KCl 4.69, $MgSO_4$ 0.59, KH_2HPO_4 1.03, $NaHPO_4$ 25, $CaCl_2$ 1.41, and glucose 11.1 (pH 7.4). The tissues were then gently flushed with cold buffer to remove blood from the lumen and were cut into 3 segments. The segments were incubated with NADPH (100 μmol/L) in buffer at 37 °C for 15 min. Lucigenin-enhanced chemiluminescence was measured in 2 mL Krebs–HEPES buffer containing lucigenin (5 μmol/L) using a Berthold FB12 single-tube luminometer, modified to maintain a sample temperature of 37 °C. Chemiluminescence was measured continuously for 15 min after allowing for dark adaptation and was expressed as relative light units per minute per milligram vessel dry weight (RLU/min/mg).

Drugs

17β-Estradiol and ICI 182,780 were obtained from Nacalai Tesque (Kyoto, Japan) and Sigma Chemical Co. (St. Louis, MO), respectively and dissolved in cottonseed oil. J-104132 and L-158809 were provided by Banyu Pharmaceutical Co., Ltd. (Tsukuba, Japan) and Merck & Co. Inc (Rahway, USA), respectively, and dissolved in distilled water. Other chemicals were purchased from Sigma Chemical Co., Nacalai Tesque (Kyoto, Japan), and Wako (Osaka, Japan).

Statistical analysis

All values were expressed as the mean ± S.E.M. Relevant data were processed by InStat (Graph-PAD Software for Science, San Diego, CA). We used a one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests for statistical analysis. Differences were considered significant at $P < 0.05$.

Results

Effects of the ERA or ARB on neointimal formation in intact female and OVX rats

Neointimal formation after the balloon injury was not observed in the uninjured arteries of all groups (data not shown).

In the injured arteries of the intact female groups, ARB, but not the ERA treatment markedly reduced the extent of neointimal formation following the balloon injury (Fig. 1A–C). The ERA treatment did not significantly decrease the neointima/media ratio after the balloon injury in intact female rats, whereas the ARB treatment did (Fig. 2). However, different results were observed in the OVX groups. The ERA treatment markedly decreased neointimal formation and the neointimal/media ratio in the injured arteries of the OVX groups (Figs. 1D, E and 3), whereas the ARB treatment did not (Figs. 1F and 3).

Effects of the combined therapy with the ERA, ARB, and 17β-estradiol on neointimal formation in OVX rats

The neointima/media ratio was significantly lower in the 17β-estradiol-treated OVX groups than in the vehicle-treated group (Fig. 3). The combined treatment with 17β-estradiol and ARB further decreased the neointima/media ratio after the balloon injury in the OVX groups, whereas no combinational effects were observed due to the combined treatment with 17β-estradiol and the ERA (Fig. 3). The combined treatment with the ERA and ARB had an additive effect on the neointima/media ratio following the balloon injury in the OVX groups.

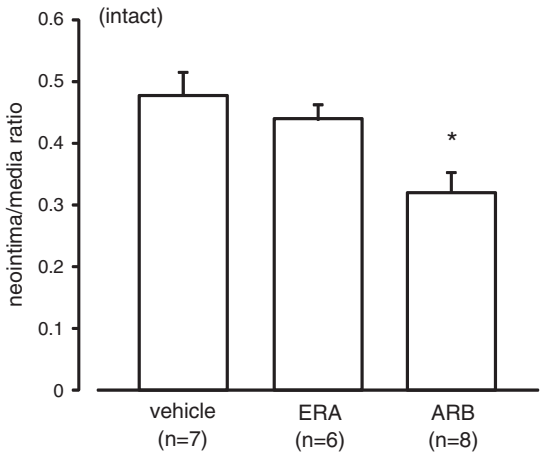

Fig. 2. Neointima/media ratio of the injured arteries in intact female rats 2 weeks after the balloon injury. Data are expressed as the mean ± S.E.M. (n = 6–8). *P < 0.05, significantly different from the vehicle-treated intact female group. ERA; endothelin receptor antagonist (10 mg/kg/day, p.o.), ARB; angiotensin receptor blocker (1 mg/kg/day, p.o.).

Effects of an estrogen receptor inhibitor on ERA or 17β-estradiol-induced inhibition of neointimal formation in OVX rats

The ERA exhibited estrogen-like vasoprotective effects on balloon injury-induced neointimal formation in OVX rats. Thus, we examined whether the vasoprotective effects of the ERA were dependent on the estrogen receptor. Treatment with ICI 182,780, an estrogen receptor inhibitor, did not significantly increase the neointima/media ratio following the balloon injury in ERA-treated OVX rats, and 17β-estradiol-induced vasoprotective actions were abolished by ICI 182,780 treatment (Fig. 3).

Effects of monotherapy or the combined therapy with the ERA, ARB, and 17β-estradiol on oxidative stress in intact and OVX rats

No significant differences were observed in NADPH-dependent superoxide production in the uninjured arteries of all groups (Fig. 4A).

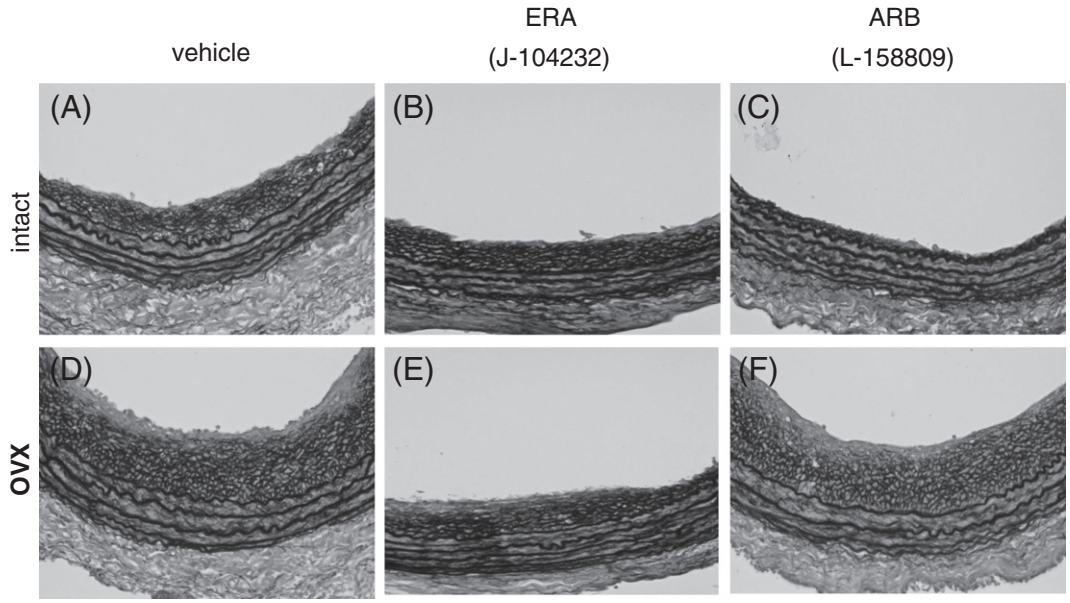

Fig. 1. Light micrographs of an injured carotid artery 2 weeks after the balloon injury in (A–C) intact female and (D–F) OVX rats. (A and D) vehicle, (B and E) ERA (J-104132; 10 mg/kg/day, p.o.), (C and F) ARB (L-158809; 1 mg/kg/day, p.o.) (Elastica Van Gieson staining, magnification ×200). ERA; endothelin receptor antagonist, ARB; angiotensin receptor blocker.

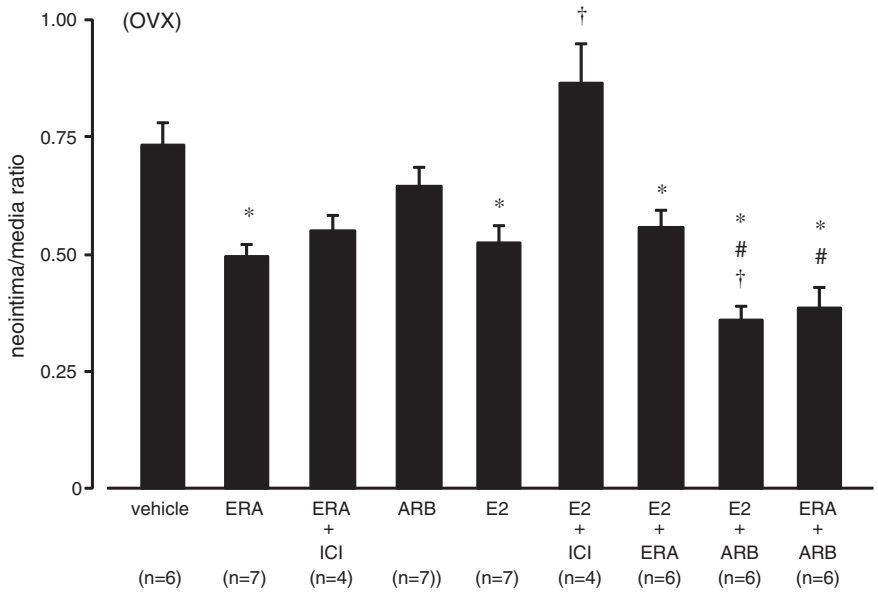

Fig. 3. Neointima/media ratio of the injured arteries in OVX rats 2 weeks after the balloon injury. Data are expressed as the mean ± S.E.M. (n = 4–7). *P < 0.05, significantly different from the vehicle-treated OVX group, #P < 0.05, significantly different from the ARB-treated OVX group, †P < 0.05, significantly different from the E2-treated OVX group. OVX; ovariectomy, ERA; endothelin receptor antagonist (10 mg/kg/day, p.o.), ARB; angiotensin receptor blocker (1 mg/kg/day, p.o.), E2; 17β-estradiol (20 μg/kg/day, s.c.), ICI; ICI 182,780 (5 mg/kg/day, s.c.).

The ARB treatment significantly suppressed NADPH-dependent superoxide production in the injured arteries of the intact female groups 2 days after the balloon injury, whereas the ERA treatment did not (Fig. 4B). On the other hand, the ERA or 17β-estradiol treatment significantly reduced NADPH-dependent superoxide production in the injured arteries of the OVX groups 2 days after the balloon injury (Fig. 4B). In contrast, the ARB treatment failed to suppress NADPH-dependent superoxide production in the injured arteries of the OVX groups (Fig. 4B). The combined treatment with the ERA or 17β-estradiol and ARB markedly suppressed NADPH-dependent superoxide production 2 days after the balloon injury in the OVX groups; however, no combinational effects were observed due to the combined treatment with the ERA and 17β-estradiol (Fig. 4B).

Discussion

ET-1 and its receptor system are known to be augmented in the vessels following vascular injury, and this system is involved in the development of vascular diseases (Douglas et al., 1994; Kirchengast and Munter, 1998; Kitada et al., in press). In addition, the ET system was shown to be at least partly responsible for the sex differences associated with vascular disease (Kitada et al., in press; Lekontseva et al., 2010; Tostes et al., 2008). On the other hand, previous studies suggested that estrogen suppresses the ET system and may contribute to the lower risk of cardiovascular diseases in premenopausal females; however, the detailed mechanism responsible remains unclear (Kitada et al., 2011; in press; Lekontseva et al., 2010; Tostes et al., 2008). Thus, the vasoprotective effects caused by ERAs in males and postmenopausal females may be superior to those in intact females because estrogen could already inhibit the augmentation of the vascular ET system following an injury in premenopausal females. We previously reported that both a selective ET_A receptor antagonist and ET_A/ET_B dual receptor antagonist significantly reduced neointimal formation following a balloon injury in male rats, but did not in intact female rats (Kitada et al., 2011). These results suggested sex differences in the vasoprotective effects of ERAs and also that ET receptor-induced neointimal formation following a balloon injury in intact female rats is less than that observed in male rats. In the present study, we demonstrated that the ERA markedly suppressed neointimal formation following a balloon injury in OVX rats, but not in intact females. This result confirmed that ERAs have superior vasoprotective effects, particularly in estrogen-deficient situations such as OVX and menopause. Thus, ERAs will be an effective tool that can replace estrogen in order to reduce the risk of vascular diseases after menopause.

OVX has been shown to cause more robust neointimal formation following vascular injury than that observed in intact female rats, and the administration of estrogen to OVX rats attenuated neointimal formation (Chen et al., 1996; Kitada et al., 2011). In addition, an estrogen receptor inhibitor aggravated neointimal formation in intact female and estrogen-treated OVX rats (Bakir et al., 2000). These results suggest that estrogen and its receptor system are an important factor for decreased neointimal formation in female rats. In the present study, the ERA exhibited estrogen-like vasoprotective effects in female rats. Therefore, we examined whether ERA-induced actions were mediated by the estrogen receptor. ICI 182,780, an estrogen receptor inhibitor, did not affect neointimal formation following the balloon injury in the ERA treatment group; however, the 17β-estradiol-induced reduction in neointimal formation was abolished by the ICI 182,780 treatment. These results suggest that ERA-induced vasoprotective effects in OVX rats are independent of the estrogen receptor, but may be closely related to downstream pathways for estrogen receptor-mediated vasoprotective effects. No combinational effects are associated with reducing neointimal formation due to the combined treatment with the ERA and 17β-estradiol. Thus, there is some overlap in the mechanisms underlying vasoprotective effects between the ERA and estrogen. However, we cannot completely deny the possibility that the vasoprotective effect of the ERA in OVX rats may have been inhibited by the estrogen receptor inhibitor because no significant difference was observed in the neointima/media ratio between OVX and OVX + ERA + ICI. The detailed mechanisms underlying ERA-induced vasoprotective effects under estrogen-deficient conditions remain unclear. An analysis of the crosstalk between estrogen and ET is needed in order to more fully understanding the pharmacological mechanisms underlying the vasoprotective actions induced by ERAs and estrogen.

Previous studies reported that ET-1 caused vascular disease *via* the induction of inflammation and oxidative stress, increases in growth factors (PDGF and FGF) and proliferative factors (EGF), and production of collagen and extracellular matrix (Bohm and Pernow, 2007; Kirchengast and Munter, 1998; Little et al., 2008; Takahashi, 2006). These ET-1-induced vascular injuries were shown to be mainly

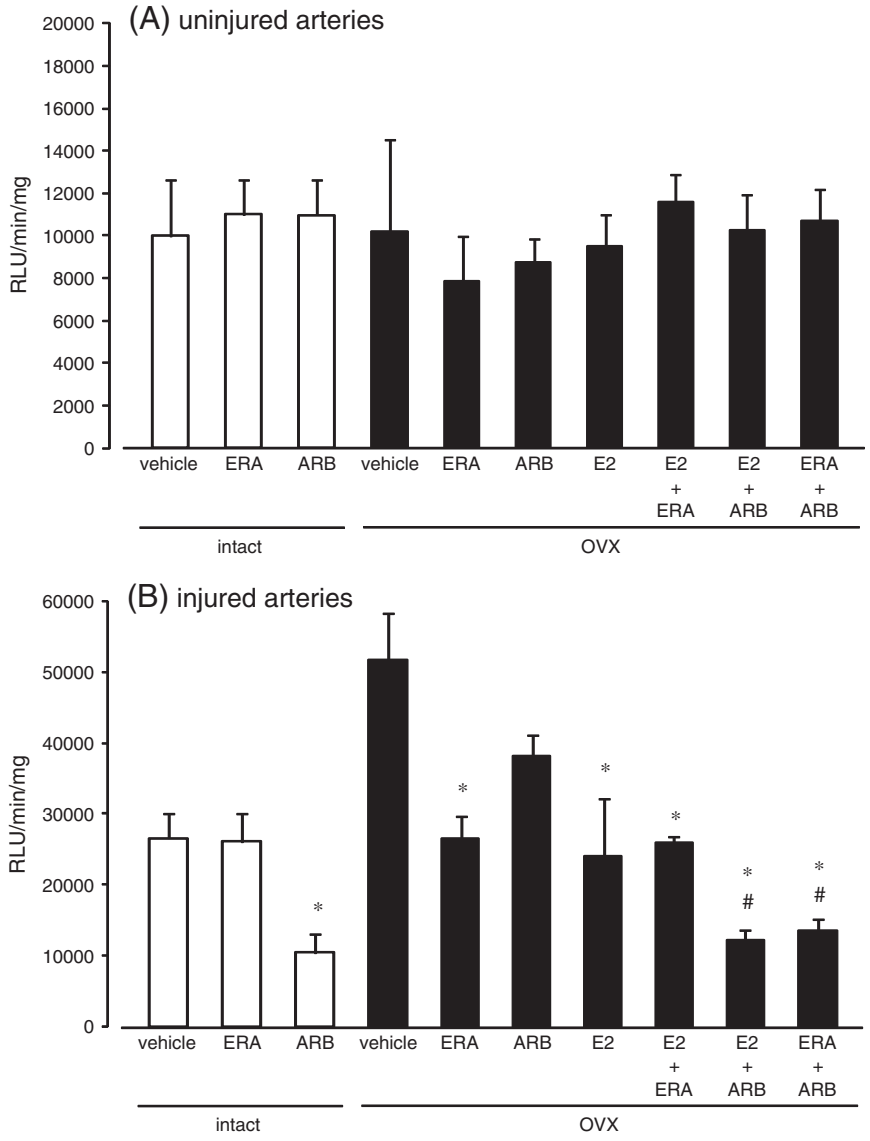

Fig. 4. NADPH-dependent superoxide production in (A) uninjured and (B) injured carotid arteries 2 days after the balloon injury. *P < 0.05, significantly different from the vehicle-treated intact or OVX group, #P < 0.05, significantly different from the ARB-treated OVX group. OVX; ovariectomy, ERA; endothelin receptor antagonist (10 mg/kg/day, p.o.), ARB; angiotensin receptor blocker (1 mg/kg/day, p.o.), E2; 17β-estradiol (20 μg/kg/day, s.c.).

mediated by ET_A and ET_B receptors (Ohkita et al., 2012). We and others demonstrated that the ET-1/ET_A receptor system plays an important role in the development of vascular disease (Douglas et al., 1994; Kitada et al., 2009; Takahashi, 2006; Wang et al., 1996). In addition, selective ET_A receptor and dual ET_A/ET_B receptor antagonists were shown to have vasoprotective effects (Douglas et al., 1994; Kitada et al., 2009; Takahashi, 2006; Wang et al., 1996). Meanwhile, understanding the pathological role of the ET-1/ET_B receptor system in vascular diseases has not been fully elucidated because of its opposing effects on endothelial and vascular smooth muscle cells; the endothelial ET_B receptor induces vasoprotective effects in injured vessels, whereas the ET_B receptor in vascular smooth muscle cells induces vascular injury (Kirkby et al., 2012; Kitada et al., 2009; Murakoshi et al., 2002; Ohkita et al., 2012). With regard to this issue, we reported that inhibiting the ET_B receptor system aggravated neointimal formation following a balloon injury in male rats and also that the chronic inhibition of ET_B receptors led to the overstimulation and/or upregulation of the ET_A receptor system (Kitada et al., 2009). These findings suggest that an augmentation in ET_A receptor-mediated ET-1 actions is a critical mechanism for the enhancement of neointimal formation observed under ET_B receptor-inhibited conditions in male rats. We also demonstrated that the inhibition of ET_B receptors in female rats abolished the sex differences associated with balloon injury-induced neointimal formation in rats (Kitada et al., 2011). Moreover, we indicated that the augmentation of ET_A receptor-mediated actions rather than ET_B receptor inhibition itself contributed to the abolition of these sex differences in ET_B receptor-inhibited rats (Kitada et al., 2011). Thus, inhibiting the ET_A receptor system is one of the critical factors in the ERA-induced prevention of neointimal formation in female as well as male rats. Although we did not investigate the effects of a selective ET_A or ET_B receptor antagonist on neointimal formation after the balloon injury in OVX rats in the present study, we believed that inhibiting the ET_A receptor system is a critical factor for the prevention of neointimal formation, irrespective of the presence or absence of ET_B receptor inhibition.

Angiotensin II is one of the most important targets in the fields of hypertension, and cardiovascular and renal diseases (Kobori et al., 2007; von Lueder and Krum, 2013). The major actions of angiotensin II are mediated by the angiotensin II type 1 (AT_1) receptor, which induces vasoconstriction, salt retention, aldosterone release, and cardiovascular and renal injuries (Kobori et al., 2007). Various basic and

clinical studies demonstrated that ARB had vasoprotective effects as well as antihypertensive actions (Hernandez Schulman et al., 2007, Nakashima et al., 2006; von Lueder and Krum, 2013). Previous studies reported that ARB and estrogen synergistically attenuated atherosclerosis and vascular remodeling following vascular injury in female mice by inhibiting oxidative stress, and ERK and STAT activities (Liu et al., 2002; Tsuda et al., 2005). We obtained similar results in the present study; ARB significantly inhibited neointimal formation following the balloon injury in intact female rats, but not in OVX rats. Furthermore, neointimal formation following the balloon injury in OVX rats was markedly less with the combined treatment with ARB and 17β-estradiol than with each monotherapy. On the other hand, one of the novel and important findings in this study is that the combined treatment with the ERA and ARB also markedly suppressed neointimal formation following the balloon injury in OVX rats, although no combinational effects were observed due to the ERA and 17β-estradiol. These results suggest that ARB exerts a strong vasoprotective effect on the estrogen-existing condition, but not on the estrogen-deficient condition and also that the ERA could replace estrogen to enhance the efficacy of ARB. Taken together, the ARB monotherapy only may not be sufficient to reduce the risk of vascular disease in women after menopause. The combined treatment with the ERA and ARB could be a new useful target for reducing the risk of vascular disease in postmenopausal women; however, further understanding of the mechanisms responsible and clinical studies on the present findings are required.

Oxidative stress plays an important role in the pathogenesis of vascular disease *via* several mechanisms such as endothelial dysfunction, inflammation, cell migration, growth, and apoptosis (Taniyama and Griendling, 2003). An increase in NADPH oxidase and this enzyme-derived reactive oxygen species were previously shown to be involved in the development of neointimal formation after vascular injury (Kurumazuka et al., 2006; Zalba et al., 2005). We previously demonstrated that the inhibition of NADPH-dependent superoxide production at an early phase after a balloon injury (2 days after the injury) was one of the critical factors for preventing neointimal formation (Kurumazuka et al., 2006). Furthermore, the attenuation of oxidative stress is known to be involved in the vasoprotective effects of estrogen (Florian et al., 2004). Thus, we hypothesize that differences in the vasoprotective effects of the ERA, ARB, 17β-estradiol, and combination therapy may be due to differences in the ability to inhibit the initial rise in NADPH-dependent superoxide production. As was observed with neointimal formation, the ERA significantly decreased NADPH-dependent superoxide production in OVX rats, but not in intact female rats, whereas ARB exhibited opposite results. These results suggest that differences in the vasoprotective effects of the ERA and ARB were at least partly due to differences in the attenuation of oxidative stress in the injured arteries of intact female and OVX rats. In addition, the combined treatment with the ERA or 17β-estradiol and ARB markedly decreased NADPH-dependent superoxide production in the injured arteries of the OVX groups. On the other hand, there were no combinational effects to decrease NADPH-dependent superoxide production observed due to the combined treatment with 17β-estradiol and the ERA, which suggests that the ERA or 17β-estradiol and ARB additively inhibit neointimal formation, and this is at least partly due to the suppression of oxidative stress. Further research to identify the detailed molecular mechanisms of these findings is warranted.

Conclusions

We demonstrated that the ERA exhibited superior vasoprotective effects under the estrogen-deficient condition than under the estrogen-existing condition, and also that the ERA, similar to estrogen, induced strong vasoprotective effects in combination with ARB. In addition, the ERA and ARB additively attenuated neointimal formation following the vascular injury, and this at least partly occurred due to the inhibition of oxidative stress. Taken together, these results suggest that ERAs may be an alternative therapy to prevent vascular disease in postmenopausal women; however, further research regarding the detailed pharmacological mechanisms of ERAs and clinical studies are needed.

Conflict of interest statement

No conflict of interest is declared by the authors.

Acknowledgments

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